Autosomal dominant polycystic kidney disease is associated with central and nephrogenic defects in osmoregulation

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Autosomal dominant polycystic kidney disease is associated with central and nephrogenic defects in osmoregulation

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Abstract

Autosomal dominant polycystic kidney disease (ADPKD) is associated with a urine-concentrating defect attributed to renal cystic changes. As PKD genes are expressed in the brain, altered central release of arginine vasopressin could also play a role. In order to help determine this we measured central and nephrogenic components of osmoregulation in 10 adults and 10 children with ADPKD, all with normal renal function, and compared them to 20 age- and gender-matched controls. Overnight water deprivation caused a lower rise in urine osmolality in the patients with ADPKD than controls, reflecting an impaired release of vasopressin and a peripheral defect in the patients. The reactivity of plasma vasopressin to water deprivation, as found in controls, was blunted in the patients with the latter showing lower urine osmolality for the same range of plasma vasopressin. The maximal urine osmolality correlated negatively with total kidney volume. Defective osmoregulation was confirmed in the children with ADPKD but was unrelated to number of renal cysts or kidney size. Thus, patients with ADPKD have an early defect in osmoregulation, with a blunted release of arginine vasopressin. This reflects expression of polycystins in hypothalamic nuclei that synthesize vasopressin, and this should be considered when evaluating treatments targeting the vasopressin pathway in ADPKD.

Keywords:

arginine vasopressin; cAMP; hypothalamus; polycystins; PKD1; PKD2; vasopressin receptor V2R

Autosomal dominant polycystic kidney disease (ADPKD) is the most frequent inherited nephropathy, characterized by the development of multiple cysts from every nephron segment. The disease progresses slowly to renal failure and end-stage renal disease in more than 70% of patients, kidney and renal cyst growth being the strongest predictors of renal function decline. Mutations in the two genes PKD1 and PKD2 account for 85 and 15% of the affected families, respectively.1 The PKD1 and PKD2 genes encode integral membrane proteins, polycystin-1 and polycystin-2, which form a complex localized in various cellular domains. The latter include the primary cilium of renal tubular epithelial cells in which the polycystins mediate calcium fluxes in response to mechanical or chemical stimuli. Mutations in PKD1/PKD2 alter intracellular calcium homeostasis and lead to cystogenesis by a combination of increased cellular proliferation, abnormal fluid secretion, and dedifferentiation.1, 2, 3

Several lines of evidence support a major role of increased concentrations of 3'-5'-cyclic adenosine monophosphate (cAMP) in the progression of renal cystic disease in ADPKD.4, 5 High cAMP levels in PKD cells lining the distal nephron can result from a reduction in intracellular Ca2+ concentration, which stimulates adenylate cyclase and inhibits phosphodiesterase activity, but also from arginine vasopressin (AVP) signaling through the vasopressin V2 receptor (V2R).1, 4 The relevance of the V2R pathway in ADPKD was demonstrated by inhibiting renal cyst development in rodent models treated with V2R antagonists or high water intake or by crossing PCK (Pkhd1-null) with AVP-null (Brattleboro) rats.1, 2, 6 These results motivated the initiation of a large, phase III clinical trial testing whether long-term use of V2R antagonists effectively slows cystogenesis, 7 and of pilot studies to evaluate the effect of water prescription in patients with ADPKD.8, 9

Thus far, the evidence of abnormal V2R-cAMP signaling in ADPKD has essentially been obtained in vitro and in rodent models, most often in relation with cystogenesis. However, early studies suggested that osmoregulation itself may be affected in ADPKD. Defective urinary concentration is frequently observed in patients with ADPKD10, 11 and is more severe in patients harboring large kidneys on ultrasound analysis.12 A peripheral resistance to AVP has been suggested, in view of higher AVP levels observed in ADPKD patients at baseline and after hypertonic saline infusion.13, 14 The usual explanation for such peripheral resistance to AVP is the existence of cystic lesions impairing establishment of the interstitial osmotic gradient driving water reabsorption in the distal nephron.1 However, impaired urine concentration has also been described before major cystic changes both in patients15 and rodent models of ADPKD.16 Furthermore, haploinsufficiency in polycystin-1 has been associated with inappropriate antidiuresis and central AVP release in a Pkd1 mouse model.17 Finally, both PKD1 and PKD2 transcripts are abundantly expressed in various regions of the brain,18 where their role(s) remain(s) unknown. Thus, the exact nature of the urinary concentrating defect in ADPKD, the potential involvement of the central release of AVP, as well as the timing of the defect and its link to cystogenesis remain open questions.

In an effort to clarify the issue, we investigated the osmoregulation parameters in two cohorts of adult and pediatric ADPKD patients. The patients were included at an early stage of disease and rigorously compared with age- and gender-matched, non-affected family members or controls. The urinary concentrating parameters were investigated at baseline and following water deprivation, in relation to plasma AVP, renal volume (measured by magnetic resonance imaging, MRI), and blood pressure. We also documented the expression of the PKD and AVP genes in the mouse and human brain. Our results show that, at an early stage, patients with ADPKD are characterized by a defective osmoregulation that involves the central release of AVP.

RESULTS

Clinical characteristics of the ADPKD patients and controls

The adult ADPKD population included seven women and three men, aged 22 to 66 years, who were matched with 10 unaffected family members (Table 1). Body weight, estimated glomerular filtration rate (eGFR), water, and NaCl intake were similar in ADPKD patients and control subjects. ADPKD patients had a significantly higher blood pressure than controls. In all, 6 of 10 adult ADPKD patients were treated with one to four antihypertensive drugs (mainly angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, diuretics, and β-blockers), whereas no control subject had hypertension.

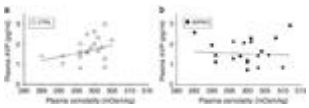
Table 1 - Clinical and biological parameters in the ADPKD patients and controls.

The children ADPKD cohort included five girls and five boys, aged 4–17 years, who were matched for age and gender with 10 non-related healthy volunteers. Body weight, body mass index, mean blood pressure, and eGFR were similar in both groups (Table 1).

Osmoregulation parameters at baseline and after water deprivation

Osmoregulation parameters (plasma and urine osmolality, plasma level of AVP, urinary cAMP levels), as well as plasma urea and creatinine and aldosterone levels were similar in ADPKD patients and controls at baseline (Table 2). Water deprivation (which induced a weight loss of ~12% in both groups) induced a higher increase in plasma osmolality in ADPKD patients than in controls (304±1.4 vs. 300±0.9 mOsm/kg, respectively, P=0.023). The maximal urine osmolality (Umax, after overnight water deprivation) was lower in ADPKD patients than in controls (710±68 vs. 920±47 mOsm/kg, respectively, P=0.020). Despite these differences in plasma osmolality, plasma AVP levels remained unchanged in ADPKD patients, contrasting with increased values in controls (Table 2). Examination of the individual parameters obtained at baseline and after water deprivation revealed that the correlation between plasma osmolality and plasma AVP, as observed in controls (Figure 1a), was blunted in ADPKD patients (Figure 1b). In contrast, the reactivity of plasma aldosterone to water deprivation was similar in both groups.

Figure 1.



Relationship between plasma osmolality and vasopressin levels in autosomal dominant polycystic kidney disease (ADPKD) and controls (CTRLs). The correlation of individual parameters at baseline

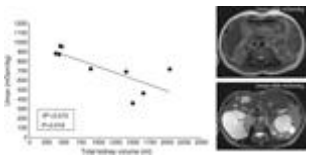
and after water deprivation shows that the positive relationship between plasma osmolality and plasma vasopressin (AVP), as observed in (a) controls, is blunted in (b) ADPKD patients.

Full figure and legend (48K)

Table 2 - Osmoregulation parameters in adult ADPKD patients and controls.

To substantiate the relationship between the severity of ADPKD and the impaired urinary concentration, we obtained total kidney volume (TKV) measurements by MRI at baseline in the cohort of adult ADPKD patients. The TKV averaged 1026 ml in the cohort (range: 450–2037 ml). Figure 2 shows that there was a negative correlation between maximal urinary concentrating ability and renal volume: patients with the largest kidneys had the most severe urinary concentrating defect.

Figure 2.



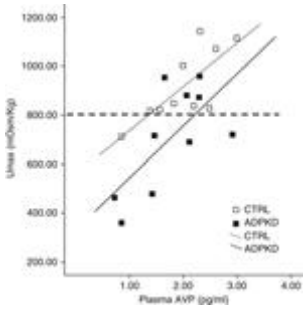
Correlation beween total renal volume and urinary concentrating ability in autosomal dominant polycystic kidney disease (ADPKD) patients. There is an inverse correlation between maximal urine concentration (Umax) and total renal volume measured by magnetic resonance imaging (MRI). The correlation is illustrated by MRI images of the kidneys of two representative patients: a 42-year-old man with few renal cysts shows a high urinary concentrating ability (959 mOsm/kg), whereas a 40-year-old woman with large cystic kidneys shows a low urinary concentrating ability (464 mOsm/kg).

Central and peripheral response to water deprivation

Osmoregulation is a complex mechanism that involves central and peripheral responses leading to the adjustment of urinary osmolality in response to changes in plasma osmolality. The combination of blunted increase in plasma AVP despite increased plasma osmolality (Table 2, Figure 1) suggested a central defect in response to water deprivation in ADPKD patients. However, the correlation between the urinary concentrating defect and kidney volume (Figure 2) suggested a peripheral component as well.

To characterize the peripheral response to AVP, we analyzed Umax as a function of plasma AVP (Figure 3). A significant correlation between plasma AVP and Umax was observed in each group, but the shift of the linear regression indicated that a higher plasma AVP level is needed to achieve the same Umax in ADPKD vs. control (CTRL; Figure 3). Furthermore, a lower proportion of ADPKD patients compared with controls (4/10 ADPKD patients vs. 9/10 controls, P=0.019) reached a Umax of 800 mOsm/kg (corresponding to standard expected value after water deprivation). These data reveal that Umax, which reflects the action of AVP on its V2R target and downstream effectors, is deficient in ADPKD. Thus, ADPKD patients present a defective peripheral response to AVP, located downstream of the V2R in the principal cells of the collecting ducts.

Figure 3.

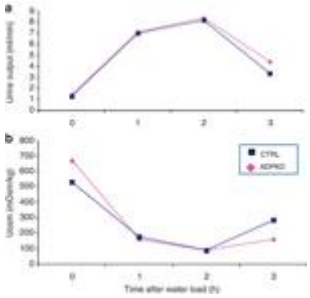


Relationship between plasma vasopressin and maximal urine osmolality in autosomal dominant polycystic kidney disease (ADPKD) patients and controls (CTRLs). The peripheral response to vasopressin was assessed by analyzing maximal urine concentration (Umax, after overnight water deprivation) as a function of plasma AVP. The dotted line represents the expected Umax in these conditions (800 mOsm/kg). Black squares represent ADPKD patients, and open squares represent CTRL subjects. The two significant regression lines in CTRL (r2: 0.58; P=0.01) and ADPKD (r2: 0.46; P=0.03) are shown, with a displacement to the right indicating that a higher plasma AVP level is needed to achieve the same Umax in ADPKD vs. CTRL. For a similar range of plasma AVP, only 4/10 ADPKD patients vs. 9/10 CTRL subjects reached a Umax >800 mOsm/kg (P=0.019).

Response to water loading

A standardized acute water loading test was performed in adult ADPKD patients and controls to assess the capacity of the kidney to dilute urine (Figure 4). The ingestion of water led to an abrupt increase in urinary output (Figure 4a), paralleled by a sharp decrease in urine osmolality (Figure 4b) during the first 2 hours of the test, with subsequent recovery during the 3 h. The kinetic and mean values of these parameters were similar in ADPKD and control groups, demonstrating that the diluting capacity was intact in these ADPKD patients.

Figure 4.

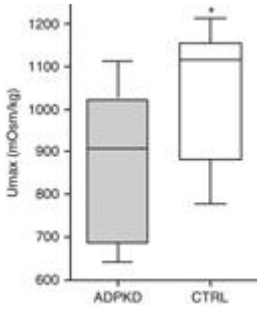


Acute water loading in autosomal dominant polycystic kidney disease (ADPKD) patients and controls (CTRLs). A similar response to acute water loading—(a) increased urine output and (b) decreased urine osmolality (Uosm)—is observed in both groups. Both the timing and magnitude of changes are similar in ADPKD vs. controls.

Osmoregulation in children with ADPKD

Children with ADPKD and controls underwent a simplified protocol to investigate osmoregulation parameters following an overnight water deprivation. The biological parameters at the end of water deprivation are shown in Table 3. As in adults, maximal urine osmolality was lower in ADPKD children than in controls (Figure 5), despite a trend for higher plasma osmolality (298±1.1 vs. 295±0.7 mOsm/kg), whereas plasma creatinine and eGFR values were similar. ADPKD children also showed lower plasma urea levels, which may suggest a decreased acute vasopressin effect on the kidney, with lower urea recirculation and less efficient urine concentration.

Figure 5.



Urine osmolality after overnight water deprivation in children with autosomal dominant polycystic kidney disease (ADPKD) and controls (CTRLs). Urine osmolality was obtained in 10 pairs of children with ADPKD vs. age- and gender-matched controls. The maximal urine osmolality (Umax) is significantly higher in CTRL than in ADPKD children (P=0.029). *P<0.05, CTRL vs. ADPKD.

Table 3 - Biological parameters after water deprivation in pediatric ADPKD patients and controls.

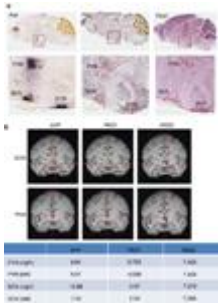
 Full table

This simplified protocol suggested that ADPKD children show both central and peripheral defects in osmoregulation. For a similar range of plasma AVP levels, 7/10 ADPKD children had a plasma osmolality higher than 296 mOsm/kg (the mean value for the two groups), whereas only 4/10 controls reached that value, suggesting a central defect into the release of AVP. For a similar range of plasma AVP levels, only 4/10 ADPKD children reached the mean maximal urine osmolality (957 mOsm/kg), whereas 7/10 control children exceeded that value. On ultrasound examination, there was no correlation between the number of renal cysts or bilateral kidney length (even normalized for height, weight, or body surface) and urinary osmolality in ADPKD children (data not shown).

Expression of PKD1 and PKD2 in osmoregulatory regions of the brain

The expression profile of PKD1 and PKD2 in the osmoregulatory regions of the brain has not been clearly demonstrated. A search of the available databases (mouse and human Allen Brain Atlas) revealed that, in comparison with the abundant Avp transcript, Pkd1 and Pkd2 are clearly enriched in the paraventricular, suprachiasmatic, and supraoptic hypothalamic nuclei of the adult mouse brain (Figure 6a). Similarly, a significant expression level of PKD1 and PKD2 was detected by microarray analysis in the AVP-expressing paraventricular and supraoptic nuceli of the hypothalamus in the human brain (Figure 6b). A significant expression of Pkd1 and Pkd2 was also identified in scattered neurons forming the subfornical organ and vascular organ of the lamina terminalis, two regions involved in thirst response (Supplementary Figure S1 online).

Figure 6.



Expression of polycystic kidney disease (PKD) genes in the hypothalamus. (a) Expression of Pkd1, Pkd2, and Avp genes (in situ hybridization) in the hypothalamic nuclei of the adult mouse. Data compiled from the Mouse Allen Brain Atlas (http://mouse.brain-map.org/). (b) Expression of PKD1, PKD2, and AVP in human brain. The expression profiles for AVP, PKD1, and PKD2 (microarray) in paraventricular nucleus (PVN) and supraoptic nucleus (SON) were obtained from Allen Brain Atlas (http://human.brain-map.org). The microarray data sets are presented as a matrix with brain

structure, using a 'heat map' format in which the colors correspond to a normalized (z-score) expression level of each probe in coronal sections of magnetic resonance imaging (MRI) space around the hypothalamus. z is negative when the raw score is below the population mean and positive when above. The data shown in the Table are log2 expression values of each probe in each brain area. Expression data of AVP: donor=10021 and probes=1058921; expression data of PKD1: donor=10021 and probes=1053025; expression data of PKD2: donor=10021 and probes=1053021. PVN, paraventricular nucleus; SCN, suprachiasmatic nucleus; SON, supraoptic nucleus.

DISCUSSION

The results presented here demonstrate that patients with ADPKD show a significant defect in osmoregulation affecting both the release of AVP in response to plasma osmolality (central component) and the V2R-mediated response (nephrogenic component). Defective osmoregulation was observed in adult and pediatric ADPKD patients early in the course of disease, whereas the capacity to dilute urine remained intact. The alteration in the central release of AVP can be explained by a significant expression of PKD1 and PKD2 together with AVP in the hypothalamic nuclei.

The existence of a urinary concentrating defect in ADPKD was first described almost 40 years ago10 and was subsequently verified in several studies.8, 11, 12, 15 The present results confirm and extend these reports, showing that the defect occurs early in the disease course as evidenced in children and adults with intact GFR, in the absence of hypokalemia or acid–base abnormalities. The extent of the urinary concentrating defect is clearly correlated with TKV as assessed by MRI in adults. In contrast, the magnitude of the defect could not be correlated with the number of renal cysts or renal length obtained by ultrasound examination in ADPKD children. The presence of cysts or their number in the early stage of ADPKD is thus not a prerequisite for the osmoregulation defect documented here. This conclusion is in fact supported by developmental studies in diphenlythiazole-induced rats16 and cpk mice,19 in which the urinary concentrating defect precedes the development of renal cysts. More than structural changes in the collecting ducts, a functional defect in cellular or interstitial processes has been evoked.19 The latter hypothesis is also supported by the altered reactivity to AVP observed in the haploinsufficient Pkd1 mouse model, which does not show renal cysts.17

Overnight water deprivation revealed that, in comparison with controls, ADPKD patients had a higher increase in plasma osmolality contrasting with a blunted release of AVP (Table 2). The relationship between plasma osmolality and AVP levels, combining data at baseline and after water deprivation, was blunted in ADPKD patients (Figure 1). In agreement with Danielsen et al.,13 baseline plasma AVP levels were similar in ADPKD and CTRL, reflecting similar plasma and urine osmolality and similar water and NaCl intake in both groups. Recently, Meijer et al.20 showed a significant correlation between plasma osmolality and copeptin, a surrogate marker of AVP, at baseline, in a cohort of ADPKD adults with CKD stages 1 to 4. The fact that plasma copeptin levels are influenced by GFR may explain why the baseline copeptin levels reported in the latter study are higher (median: 7.0 pmol/l) than in controls from the PREVEND study (median: 4.7 pmol/l).21 This could possibly also explain the correlation between plasma copeptin and osmolality because of the contribution of urea to measured osmolality. Importantly, no healthy controls were investigated by Meijer et al.,20 and the reactivity to water deprivation was also not tested. Taken together, these elements suggest that ADPKD patients have a central defect altering the release of AVP in response to increased plasma osmolality levels caused by water deprivation.

What could be the molecular counterpart of such a central defect? ADPKD is not classically associated with functional defects in the central nervous system. However, both PKD1 and PKD2 genes show a predominant neural expression during development, with high expression levels (particularly for PKD1) still detected in the adult brain.18 Similarly, the Pkd1 transcript has been detected in intracranial arteries,22 and polycystin-1 has been shown to be expressed in ciliated structures in the lateral and third ventricles of the mouse brain.23 Data extracted from the Allen Brain Atlas show that, in fact, both Pkd1/Pkd2 (mouse) and PKD1/PKD2 (human) transcripts are expressed in the supraoptic, suprachiasmatic, and paraventricular nuclei that synthesize and release AVP in response to a rise in plasma osmolality (Figure 6).

Intrinsic osmosensitivity of the vasopressin-containing neurons is conferred by mechanosensitive cation channels that include TRPV1 and TRPV4.24 There is evidence that polycystin-2 interacts with TRPV4 to form a mechanosensor driving calcium transients in vitro.25 It is interesting to note that mice lacking TRPV4 show a lower increase in circulating AVP upon hypertonic stress while being more hyperosmolar than wild-type littermates,26 i.e., a similar phenotype to that of the ADPKD patients examined here. Recently, it was shown that Trpv4-null mice also show defective activation of an osmosensitive sensory system in the liver.27 Although the exact roles of TRPV1 and TRPV4 in hypertonicity sensing remain debated,28 one could hypothesize that a defect in the multiprotein complex transducing calcium-dependent information in vasopressin neurons could be defective in ADPKD patients. Alternatively, the functional loss of polycystins could affect the level of AVP in hypothalamic neurons, or interfere with the clock control of AVP release in the late sleep period.29 A potential role of the polycystins in thirst could also be suggested, based on the detection of their transcripts in the subfornical organ and vascular organ of the lamina terminalis regions of the mouse brain. At any rate, this extrarenal manifestation of ADPKD suggests a role for polycystins in the hypothalamus. This could be relevant for the use of vasopressin antagonists, known to be associated with increased levels of endogenous AVP.30

In addition to the central component, the ADPKD patients investigated here show manifestations of peripheral resistance to AVP. Maximal urine osmolality after water deprivation was consistently lower, despite a similar range of plasma AVP. The shifted relationship between plasma AVP and Umax in ADPKD patients and the lower proportion of patients reaching a Umax value >800 mOsm/kg concur with this conclusion. In addition, the magnitude of the concentration defect was significantly correlated with TKV obtained by MRI. The exact nature of the peripheral defect in AVP-V2R signaling in ADPKD kidneys remains uncertain. In the absence of cAMP measurement in the renal papilla, the value of urinary cAMP as an indicator of the V2R response is questionable, as this parameter may essentially reflect the activity of PTH in the proximal tubule.31

On the basis of evidence suggesting that increased cAMP levels are involved in cyst progression, the ingestion of supplemental water is increasingly considered as a potential treatment for ADPKD.6 A normal capacity to dilute urine was observed in two preliminary studies evaluating the effect of water prescription in a total of 21 ADPKD patients with preserved eGFR (>60 ml/min per 1.73 m2).8, 9 Our report of an intact urinary diluting ability over a 3-h follow-up in ADPKD patients exposed to an acute water load supports these results. Furthermore, the detailed kinetics suggest that the rapid lowering of the circulating AVP is totally preserved in ADPKD patients (Figure 4). Hence, the central defect discussed above indeed concerns the coupling between increased plasma osmolality and release of AVP by hypothalamic neurons.

Several limitations of our study should be acknowledged. First, the study population is small and selected, with limited statistical power. However, we feel that the rigorous investigation protocol and the inclusion of age- and gender-matched controls (with unaffected siblings for the adult population) bring an added value to the analysis. Second, osmoregulatory parameters were measured once at baseline, and thus we could not assess the effect of variation over time. The facts that the background noise is greater with a single measure and that the measure was obtained in exactly similar conditions in both groups, before and after reactivity testing, rather support the relevance of our data. Third, we did not use the method of acute hypertonic saline injection to assess the central release of AVP. Instead, the use of a controlled water deprivation test was considered more physiological and less invasive in a population that included a majority of hypertensive patients deprived from their antihypertensive medications for 3 weeks at the time of investigation. Ethical considerations were also guiding the choice of a much simplified protocol in the children cohort. Additional studies will thus be necessary to address these limitations and confirm our findings.

In conclusion, the present study shows that patients with ADPKD show both a central and peripheral defect in osmoregulation early in the course of the disease. The central defect, which parallels the expression of PKD genes in hypothalamic neurons that synthesize and release AVP, is a novel extrarenal manifestation of ADPKD. These data provide insights into the role of polycystins in the brain and are relevant when considering treatments targeting AVP in ADPKD.

MATERIALS AND METHODS

Patients and controls

Twenty ADPKD patients, 10 adults and 10 children, with a normal renal function (eGFR $\geqslant$ 60 ml/min) were included in the study. The clinical diagnosis of ADPKD was assigned according to the ultrasonographic criteria of Ravine32 in patients with a familial history of ADPKD. Controls for the ADPKD adults were age- and gender-matched non-affected members of ADPKD families, in which the disease had been excluded by negative renal ultrasonography after the age of 30 years or by linkage analysis. Controls for the ADPKD children were unrelated healthy children, matched for age and gender, with no familial history of renal disease. The study protocol has been approved by the Ethics Committee of the UCL Medical School, Brussels; adult study subjects or the parents of the pediatric study subjects gave written informed consent.

Investigation protocol in adults and children

Adult patients with ADPKD and controls underwent a screening visit 3 to 4 weeks before a baseline evaluation that was immediately followed by an overnight (16 h) water deprivation test and an acute water loading test over a period of 2 days. Patients taking angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, or diuretics stopped these medications 3 weeks before the baseline visit. Subjects were allowed to eat normally during the investigations. Clinical parameters, urine, and blood samples were collected at baseline and at the end of the water deprivation. The water loading test consisted of the ingestion of 20 ml/kg body weight tap water in 30 min, followed by the hourly determination of urine flow and urine osmolality for the next 3 h. TKV was determined by magnetic resonance imaging (Achieva 3.0T TX MRI, Philips Healthcare, Best, The Netherlands).

For ethical and technical reasons, the protocol was considerably simplified in children. Children taking angiotensin-converting enzyme inhibitors, angiotensin receptor blockers or diuretics were excluded from the study, because interruption or modification in their treatment was not considered feasible. Children had no baseline evaluation, but only one visit to collect blood and urine samples at the end of a 12-h overnight water deprivation. Kidney length and the number of cysts were determined by renal ultrasonography (ATL IU22 Ultrasound Machine, Philips Healthcare) in ADPKD children.

Clinical and biological analyses

Blood pressure, body weight, urine output, plasma, and urine osmolality were assessed at baseline, after water deprivation, and during the water loading test. Urinary concentrating ability was assessed by maximal urine osmolality (Umax) reached at the end of water deprivation. Plasma levels of urea, creatinine, sodium, potassium, chloride, calcium, and proteins were measured at baseline and after water deprivation, using routine laboratory analyses (Synchron CX5, Beckman-Coulter, Fullerton, CA). The eGFR was calculated using the Cockcroft formula in adults, and by the Schwartz adapted formula33 in children. The concentrations of creatinine, sodium, and protein were measured on 24-h urine collections in adults, and on a second morning fasting urine sample in children. Plasma and urine osmolality was measured using a Fiske Osmometer (Needham Heights, MA). Plasma levels of AVP were determined in duplicate by radioimmunoassay34 (Tenon Hospital, Paris, France). Plasma levels of renin were determined by enzyme immunoassay (Liaison automate, DiaSorin, Anderlecht, Belgium), plasma aldosterone by radioimmunoassay (TKAL2, Siemens, Brussels, Belgium), and urinary cyclic AMP by enzyme immunoassay (Sigma-Aldrich, Bornem, Belgium).

AVP and PKD gene expression in mouse and human brain

The expression pattern of mouse (Pkd1, Pkd2, and Avp) and human (PKD1, PKD2, and AVP) genes was obtained from the appropriate sections of the Allen Brain Atlas (http://mouse.brain-map.org/ and http://human.brain-map.org/, respectively).

Statistical analysis

Data are means±standard error of the mean (s.e.m.). Comparisons between groups were performed using two-tailed unpaired t-test. Bivariate correlation and $\chi 2$ test were used for parameter correlations. All statistical analyses were performed using the SPSS 15.0 software (Brussels, Belgium). All tests were two-tailed, and $P<0.05$ was considered significant.

Disclosure

All the authors declared no competing interests.

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